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The vast majority of the more than 1 billion doses of vaccines manufactured worldwide each year are given to perfectly healthy people.<sup>1–4</sup> It is this fact that drives the requirements for vaccines to be among the most rigorously designed, monitored, and compliant products manufactured today. The ability to manufacture these vaccines safely and consistently is built on four competencies:

1. The manufacturing process that defines how the product is made;
2. The compliance of the organization to successfully complete that process;
3. The testing of the product and supporting operations; and
4. The regulatory authorization to release and distribute the product.

This chapter examines how each of these components is established during the development of a new vaccine and how the field of vaccine manufacturing is responding to emerging challenges for increased capacity (e.g., pandemic influenza vaccine), increased safety assurance (e.g., barrier isolator filling), and increasing complexities of manufacture (e.g., conjugate vaccines). All of this must be accomplished while consistently delivering more than 1 billion doses annually at the relatively low cost of similar therapeutic products.

In the United States, vaccines are regulated as biological products. The U.S. Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research (CBER) is responsible for regulating vaccines. Current authority for the regulation of vaccines resides primarily in Section 351 of the Public Health Service Act and specific sections of the Federal Food, Drug and Cosmetic Act.<sup>5,6</sup> Section 351 of the Public Health Service Act gives the federal government the authority to license biological products and the establishments where they are produced.<sup>7</sup> Vaccines undergo a rigorous review of laboratory, nonclinical, and clinical data to ensure safety, efficacy, purity, and potency. Vaccines approved for marketing may also be required to undergo additional studies to further evaluate the vaccine and often to address specific questions about the vaccine's safety, effectiveness, or possible side effects.<sup>8</sup>

In the European Union, animal and human vaccines are regulated by the European Medicines Agency (EMA), whose main responsibility is the promotion of public and animal health. The EMA's Committee on Medicinal Products for Human Use through its Vaccine Working Party has oversight for human vaccines. Vaccines are licensed through a centralized procedure that allows for simultaneous licensure within all countries within the European Union. Human vaccines manufacturing is regulated under a Good Manufacturing Practices (GMP) Directive 200/94/EEC, Annex 16, and Annex 2.

Harmonization of licensing and regulating procedures for vaccines worldwide has obvious benefits in rapidly delivering safe and effective vaccines to the market. Impediments to harmonization include lack of standardized regulatory procedures and mutual recognition of licenses and inspections between countries and worldwide regulatory agencies. Harmonization of regulation continues to progress as joint FDA-EMA establishment inspections programs have become a

reality and adherence to International Conference on Harmonisation (ICH) guidance is expected.

New vaccines are subjected to a well-defined regulatory process for approval. The approval process consists of four principal elements:

- Preparation of preclinical materials for proof-of-concept testing in animal models; manufacture of clinical materials according to current GMP; and toxicology analysis in an appropriate animal system.
- Submission of an investigational new drug (IND) application for submission to FDA for review.
- Testing for safety and effectiveness through clinical and further nonclinical studies (Phase I to Phase III clinical studies).
- Submission of all clinical, nonclinical, and manufacturing data to the FDA and EMA in the form of a Biologics License Application (BLA) for final review and licensure.

This chapter outlines the basics of manufacturing a vaccine and a description of some examples of currently licensed products. It then moves to the regulatory requirements for vaccine manufacturing including current GMP compliance, and then discusses the development of new vaccines. The final section examines the great challenges in the field to deliver a product held to an ever-increasing standard of safety while providing sufficient doses at reasonable costs for an ever-increasing number of diseases.

## MANUFACTURING BASICS

The manufacture of vaccines is composed of several basic steps that result in the finished product. [Table 5.1](#) summarizes these steps with examples for pathogens that have a licensed vaccine. The first step is the generation of the antigen used to induce an immune response. This step includes the generation of the pathogen itself (for subsequent inactivation or isolation of a subunit) or generation of a recombinant protein derived from the pathogen. Vaccines under development use additional methods that will be discussed later. Viruses are grown in cells, which can be either primary cells, such as chicken fibroblasts (e.g., yellow fever vaccine), or continuous cell lines, such as MRC-5 (e.g., hepatitis A vaccine). Bacterial pathogens are grown in bioreactors using medium developed to optimize the yield of the antigen while maintaining its integrity. Recombinant proteins can be manufactured in bacteria, yeast, or cell culture. The viral and bacterial seed cultures and the cell lines used for viral production are carefully controlled, stored, characterized, and, often, protected. The first step in manufacture is the establishment of a "master cell bank." This is a collection of vialled cells that form the starting material for all future production. It is extensively characterized for performance and the absence of any adventitious agents. From this bank, working cell banks are prepared that are used as the routine starting culture for production lots. The final vaccine is a direct function of its starting materials, and a change in this seed can be as complicated as initiating a new product development altogether.

TABLE 5.1 Examples of Licensed Vaccine Manufacturing Processes

Disease	Trade Name	Generic Name	Cell Culture/Fermentation	Isolation	Purification	Formulation	Preservative
Anthrax	BioThrax	Anthrax Vaccine Adsorbed	Chemically defined protein-free media growing a microaerophilic culture of avirulent, nonencapsulated <i>Bacillus anthracis</i>	ND	Sterile filtrate of culture medium	Aluminum hydroxide	Benzethonium and formaldehyde
<i>Haemophilus influenzae</i>	ActHIB	<i>Haemophilus b</i> Conjugate Vaccine (Tetanus Toxoid Conjugate)	Grown of <i>Haemophilus influenzae</i> type b strain 1482 grown in a semisynthetic medium	Centrifugation	Phenol extraction and alcohol precipitation; Hib polysaccharide conjugated to tetanus toxoid	Lyophilized	None
Hepatitis A	Havrix	Hepatitis A Vaccine, Inactivated	Hepatitis A (strain HM175) propagated in MRC-5 human diploid cells	Cells lysed to form a suspension	Purification by ultrafiltration and gel permeation chromatography followed by formalin inactivation	Adsorbed onto aluminum hydroxide	2-Phenoxy-ethanol
Hepatitis B	Recombivax HB	Hepatitis B Vaccine (recombinant)	Recombinant hepatitis B surface antigen (HBsAg) produced in yeast cells grown in a complex medium of extract of yeast, soy peptone, dextrose, amino acids, and mineral salts	Released from yeast by cell disruption	Series of chemical and physical methods (ND) followed by treatment with formaldehyde	Coprecipitation of HBsAg with amorphous aluminum hydroxyphosphate sulfate	None
Influenza	Fluzone	Inactivated Influenza Virus Vaccine	Propagation on embryonated chicken eggs	Low-speed centrifugation and filtration	Purification/concentration on linear sucrose density gradient using continuous flow centrifugation followed by additional purification by chemical means	Phosphate-buffered saline with gelatin as stabilizer	Thimerosal in some package configurations
Japanese encephalitis	JE-VAX	Japanese Encephalitis Virus Vaccine Inactivated	Intracerebral inoculation of mice	Harvest of brain tissue/homogenization	Centrifugation, supernatant collection followed by formaldehyde inactivation; further purification by ultracentrifugation through 40% sucrose	Lyophilized	Thimerosal
Measles, mumps, rubella, and varicella	ProQuad	Measles, Mumps, Rubella and Varicella (Oka/Merck) Virus Vaccine Live	Measles virus propagated in chick embryo cell culture; mumps virus in chick embryo cell culture; rubella virus propagated in WI-38 human diploid lung fibroblasts; varicella virus propagated on MRC-5 cells	ND	ND	Lyophilized	None

Meningococcal	Menactra	Meningococcal (groups A, C, Y, and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine	Meningococcal strains are cultured individually on Mueller-Hinton agar and grown in Watson-Scherp media; <i>Corynebacterium diphtheriae</i> grown on modified Mueller and Miller medium	Extraction of polysaccharide from cell	Polysaccharide purified by centrifugation, detergent precipitation, alcohol precipitation, solvent extraction, and diafiltration; purified by ammonium sulfate fractionation and diafiltration; conjugate purified by serial diafiltration	Sodium phosphate-buffered isotonic sodium chloride	None
Pneumococcal	Prevnar	Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM197 Protein)	<i>Streptococcus pneumoniae</i> serotypes 1, 3, 4, 5, 6A, 6B, 7 F, 9 V, 14, 18 C, 19A, 19 F, and 23 F individually grown on soy peptone broth; <i>C. diphtheriae</i> strain containing CRM197 grown in caseamino acids and yeast extract-based medium	Polysaccharides isolated by centrifugation; CRM197 ND	Polysaccharides purified by precipitation, ultrafiltration, and column chromatography; CRM197 purified by ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography; conjugation done by reductive amination and the conjugate purified by ultrafiltration and column chromatography	Aluminum hydroxide suspension	None
Polio	IPOL	Poliovirus Vaccine Inactivated	Types 1, 2, and 3 poliovirus individually grown in Vero cells on microcarriers using Eagle MEM modified medium supplemented with newborn calf serum	Clarification (method ND) and concentration	Purification by three chromatography steps: anion exchange, gel filtration, and anion exchange; inactivation by formalin	Medium M-199	2-Phenoxy-ethanol
Rabies	RabAvert	Rabies Vaccine	Rabies virus grown in primary culture of chicken fibroblasts in synthetic cell culture medium with the addition of human albumin, polygeline, and antibiotics	Inactivated with β-propiolactone	Purification by zonal centrifugation in a sucrose density gradient	Stabilized with buffered polygeline and potassium glutamate; lyophilized	None
<i>Streptococcus pneumoniae</i>	Pneumovax	Pneumococcal vaccine polyvalent	ND	ND	ND	Isotonic saline	Phenol
Typhoid fever	Vivotif	Typhoid Vaccine Live Oral Ty21a	Fermentation using medium containing a digest of yeast extract, an acid digest of casein, dextrose, and galactose	Centrifugation	ND	Enteric-coated capsule containing lyophilized product	None
Yellow fever	YF-Vax	Yellow Fever Vaccine	Strain 17D-204 of yellow fever is cultured on living avian leukosis virus-free chicken embryos	Homogenization	Centrifugation	Lyophilized product containing gelatin and sorbitol as stabilizer	None
Data from vaccine package inserts. ND, not disclosed.							

The next step is to release the antigen from the substrate and isolate it from the bulk of the environment used in its growth. This can be isolation of free virus or of secreted proteins from cells or of cells containing the antigen from the spent medium. The next step is purification of the antigen. For vaccines that are composed of recombinant proteins, this step may involve many unit operations of column chromatography and ultrafiltration. For an inactivated viral vaccine, there may simply be inactivation of isolated virus with no further purification. The formulation of the vaccine is designed to maximize the stability of the vaccine while delivering it in a format that allows efficient distribution and preferred clinical delivery of the product. The formulated vaccine may include an adjuvant to enhance the immune response, stabilizers to prolong shelf life, and/or preservatives to allow multidose vials to be delivered.

Formulation consists of combining all components that constitute the final vaccine and uniformly mixing them in a single vessel (Fig. 5.1). Operations are conducted in a highly controlled environment with employees wearing special protective clothing to avoid contamination with adventitious agents. Control monitoring of the environment and critical surfaces is conducted during operations. Quality control (QC) testing at this stage usually consists of safety, potency, purity, sterility, and other assays specific to the product.

During this phase, individual, scrupulously cleaned, depyrogenated, single-dose or multidose containers are filled with vaccine and sealed with sterile stoppers or plungers. If the vaccine is to be lyophilized, the vial stoppers are inserted only partially to allow moisture to escape during the lyophilization process, and the vials are moved to a lyophilization chamber. All vials receive outer caps over the stopper for container closure integrity. To preclude the introduction of extraneous viable and nonviable contamination, all filling operations must take place in a highly controlled environment where people, equipment, and components are introduced into the critical area in a controlled manner. After filling, all containers are inspected using semiautomated or automated equipment designed to detect any minute cosmetic and physical defects. As with the formulation phase of the vaccine manufacturing operation, extensive control and monitoring of the environment and critical surfaces are conducted during operations. QC testing at this stage also consists of safety, potency, purity, sterility, and other assays that may be specific to the product.

Vaccine efficacy can be adversely affected by improper distribution and storage conditions. The sensitivity of vaccines to

adverse environmental conditions, particularly temperature extremes, varies depending on their composition. Live attenuated vaccines tend to be more susceptible than inactivated vaccines and toxoids.<sup>1</sup> Vaccines are formulated such that the potency at the end of shelf-life remains above the effective dose demonstrated in human clinical trials. As the product may degrade over the 2 to 3 years of shelf life, the release target potency may be significantly above the specified end-of-shelf-life specification. This “overformulation” can represent a significant production yield loss and cost-of-goods increase for the final product in order to support the necessary lead times to deliver and store the vaccines, especially if they are used as a rotating stockpile to protect against supply interruption or an emergency use that does not materialize. The addition of stabilizers or lyophilization, when feasible, tends to improve the thermal resistance of vaccines. Storage at very low temperatures within the manufacturing supply chain may be used to reduce potency loss during storage.

Although recommended storage conditions for many vaccines have been detailed,<sup>9</sup> the vaccine manufacturers are responsible for developing data before and after licensing that demonstrate the stability of their vaccines under recommended storage conditions for the claimed shelf life. Generally, these programs provide data in excess of the claimed shelf life (up to 3 years) to support the development of new products intended for clinical use, as well as routine support of currently marketed products, expiration date extension, and supporting distribution conditions.<sup>10,11</sup> Accelerated studies conducted at elevated temperatures are commonly applied to better understand the impact of transient temperature excursions on the vaccine. Manufacturers are required to assure that products under their control are maintained under appropriate conditions so that the identity, strength, quality, and purity of the products are not affected.<sup>12</sup>

Currently, only a limited number of vaccines are required by federal regulation to have specified shipping temperatures.<sup>10</sup> Although most vaccine manufacturers use insulated containers and other precautions for the brief (usually 24 to 72 hours) shipping time, occasional, unanticipated temperature excursions may occur that could have a detrimental impact on the shipped product. Before accepting any vaccine shipment, users should look for any evidence of improper transportation conditions, including excessive transport time and possible adverse ambient temperature conditions.<sup>1</sup>

## EXAMPLES OF VACCINE PRODUCTION

### Inactivated Virus (Influenza)

Influenza virus vaccine for intramuscular use is a sterile suspension prepared from influenza viruses propagated in chicken embryos. This vaccine is the primary method for preventing influenza and its more severe complications.<sup>13</sup>

Typically, influenza vaccine contains two strains of influenza A viruses (H1N1 and H3N2) and a single influenza B virus. An additional strain of the influenza B virus was added, with the first four-antigen-containing-vaccine licensed in 2012.<sup>14</sup> The two type A viruses are identified by their subtypes of hemagglutinin and neuraminidase. The hemagglutinin and neuraminidase glycoproteins of influenza A virus comprise the major surface proteins and the principal immunizing antigens of the virus. These proteins are inserted into the viral envelopes as spike-line projections in a ratio of approximately 4:1.<sup>15</sup>

The trivalent subunit vaccine is the predominant influenza vaccine used today. This vaccine is produced from viral strains that are identified early each year by the World Health Organization, the Centers for Disease Control and Prevention

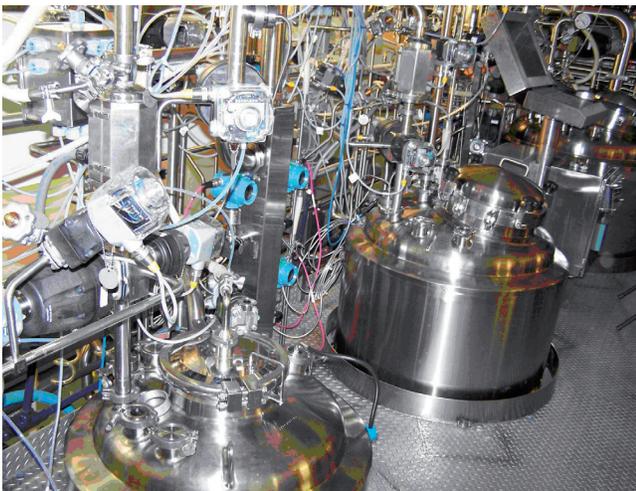


Figure 5.1. Automated vaccine formulation vessels.

(CDC), and CBER. For U.S.-licensed manufacturers, the viral strains are normally acquired from CBER or CDC. European strains are typically provided by the National Institute for Biological Standards and Control, and Southern Hemisphere strains by the Therapeutic Goods Administration of Australia. These viral strains are used to prepare cells banks at each manufacturer, which cell banks are ultimately used as the inoculums for vaccine production.

The substrate most commonly used by producers of influenza vaccine is the 11-day-old embryonated chicken egg. A monovalent virus (suspension) is received from CBER or the CDC. The monovalent virus suspension is passed in eggs. The inoculated eggs are incubated for a specific time and temperature regimen under controlled relative humidity and then harvested. In the European Union, the number of passages from the original sample is limited. The harvested allantoic fluids, which contain the live virus, are tested for infectivity, titer, specificity, and sterility. These fluids are then stored wet frozen at extremely low temperatures to maintain the stability of the monovalent seed virus (MSV).<sup>16</sup> This MSV is also certified by CBER.

Once the MSV is introduced into the egg by automated inoculators, the virus is grown at incubated temperatures, and then the allantoic fluid is harvested and purified by high-speed centrifugation on a sucrose gradient or by chromatography. The purified virus is often split using a detergent before final filtration. The virus is inactivated using formaldehyde before or after the primary purification step, depending on the manufacturer. This is repeated for three or four strains of virus, and the individually tested and released inactivated viral concentrates are combined and diluted to final vaccine strength. Fig. 5.2 outlines the overall process.

The inactivated virus vaccine described above is used for the majority of flu vaccine produced and sold today. In recent years, the inactivated influenza vaccine produced on mammalian cell culture has been approved in a number of countries. The process replaces the egg-based virus expansion with a certified cell line; the downstream processes are similar, but focused on removing the host cell protein and DNA to below designated thresholds. A recombinant influenza vaccine, produced in insect cells infected with a recombinant baculovirus to express the hemagglutinin protein has also been approved in the United States.

## Recombinant Protein (Hepatitis B)

In July 1986, a recombinant hepatitis B vaccine was licensed in the United States. This vaccine built on the knowledge that heat-inactivated serum containing hepatitis B virus (HBV) and hepatitis B surface antigen (HBsAg) was not infectious, but was immunogenic and partially protective against subsequent exposure to HBV.<sup>17</sup> HBsAg was the component that conferred protection to HBV on immunization.<sup>18</sup> To produce this vaccine, the gene coding for HBsAg, or “S” gene, was inserted into an expression vector that was capable of directing the synthesis of large quantities of HBsAg in *Saccharomyces cerevisiae*. The HBsAg particles expressed by and purified from the yeast cells have been demonstrated to be equivalent to the HBsAg derived from the plasma of the blood of hepatitis B chronic carriers.<sup>17,19,20</sup>

The recombinant *S. cerevisiae* cells expressing HBsAg are grown in stirred tank fermenters. The medium used in this process is a complex fermentation medium that consists of an extract of yeast, soy peptone, dextrose, amino acids, and mineral salts. In-process testing is conducted on the fermentation product to determine the percentage of host cells with the expression construct.<sup>7</sup> At the end of the fermentation process, the HBsAg is harvested by lysing the yeast cells. It is

separated by hydrophobic interaction and size-exclusion chromatography. The resulting HBsAg is assembled into 22-nm-diameter lipoprotein particles. The HBsAg is purified to greater than 99% for protein by a series of physical and chemical methods. The purified protein is treated in phosphate buffer with formaldehyde, sterile filtered, and then coprecipitated with alum (potassium aluminum sulfate) to form bulk vaccine adjuvanted with amorphous aluminum hydroxyphosphate sulfate. The vaccine contains no detectable yeast DNA but may contain not more than 1% yeast protein.<sup>7,19,21</sup> In a second recombinant hepatitis B vaccine, the surface antigen expressed in *S. cerevisiae* cells is purified by several physiochemical steps and formulated as a suspension of the antigen adsorbed on aluminum hydroxide. The procedures used in its manufacturing result in a product that contains no more than 5% yeast protein. No substances of human origin are used in its manufacture.<sup>20</sup> Vaccines against hepatitis B prepared from recombinant yeast cultures are noninfectious<sup>20</sup> and are free of association with human blood and blood products.<sup>19</sup>

Each lot of hepatitis B vaccine is tested for safety, in mice and guinea pigs, and for sterility.<sup>19</sup> QC product testing for purity and identity includes numerous chemical, biochemical, and physical assays on the final product to assure thorough characterization and lot-to-lot consistency. Quantitative immunoassays using monoclonal antibodies can be used to measure the presence of high levels of key epitopes on the yeast-derived HBsAg. A mouse potency assay is also used to measure the immunogenicity of hepatitis B vaccines. The effective dose capable of seroconverting 50% of the mice (ED<sub>50</sub>) is calculated.<sup>21</sup>

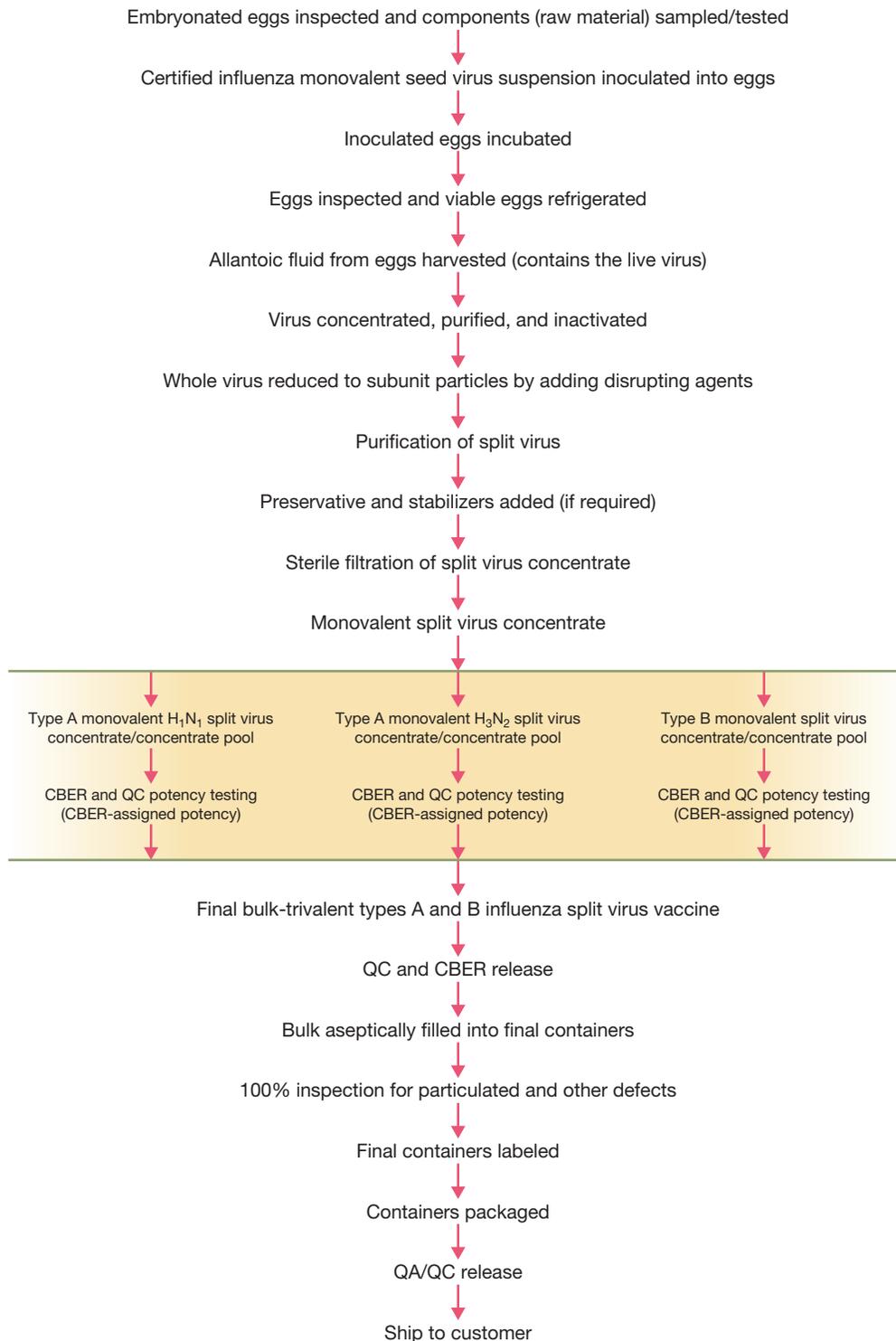
Hepatitis B vaccines are sterile suspensions for intramuscular injection. The vaccine is supplied in four formulations: pediatric, adolescent/high-risk infant, adult, and dialysis.

All formulations contain approximately 0.5 mg of aluminum (provided as amorphous aluminum hydroxyphosphate sulfate) per milliliter of vaccine.<sup>19</sup> Table 5.2 summarizes the QC testing requirements for the release of recombinant hepatitis B vaccine.

Most vaccines are still released by CBER on a lot-by-lot basis; but for several extensively characterized vaccines, such as hepatitis B and human papillomavirus (HPV) vaccines, which are manufactured using recombinant DNA processes, this requirement has been eliminated. Their manufacturing process includes significant purification, and they are extensively characterized by their analytical methods. In addition, hepatitis B vaccine had to demonstrate a “track record” of continued safety, purity, and potency to qualify for this exemption.<sup>7,22</sup>

**TABLE 5.2** Testing Requirements for the Release of Recombinant Hepatitis B Vaccine

Type of Test	Stage of Production
Plasmid retention	Fermentation production
Purity and identity	Bulk-adsorbed product or nonadsorbed bulk product
Sterility	Final bulk product
Sterility	Final container
General safety	Final container
Pyrogen	Final container
Purity	Final container
Potency	Final container



**Figure 5.2. Egg-based influenza vaccine manufacturing process flow.** CBER, Center for Biologics Evaluation and Research (of the U.S. Food and Drug Administration); QA, quality assurance; QC, quality control.

### Conjugate Vaccine (*Haemophilus influenzae* Type B)

The production of *Haemophilus influenzae* type b (Hib) conjugate includes the separate production of capsular polysaccharide from Hib and a carrier protein such as tetanus protein from *Clostridium tetani* (i.e., purified tetanus toxoid), CRM

protein from *Corynebacterium diphtheriae*, or outer membrane protein complex of *Neisseria meningitidis*.

The capsular polysaccharide is produced in industrial bioreactors using approved seeds of Hib. A crude intermediate is recovered from fermentation supernatant, using a cationic detergent. The resulting material is harvested by continuous-flow centrifugation. The paste is then resuspended in buffer,

and the polysaccharide is selectively dissociated from disrupted paste by increasing the ionic strength. The polysaccharide is then further purified by phenol extraction, ultrafiltration, and ethanol precipitation. The final material is precipitated with alcohol, dried under vacuum, and stored at  $-35^{\circ}\text{C}$  for further processing.

Tetanus protein is prepared in bioreactors using approved seeds of *C. tetani*. The crude toxin is recovered from the culture supernatant by continuous-flow centrifugation and diafiltration. Crude toxin is then purified by a combination of fractional ammonium sulfate precipitation and ultrafiltration. The resulting purified toxin is detoxified using formaldehyde, concentrated by ultrafiltration, and stored at between  $2^{\circ}\text{C}$  and  $8^{\circ}\text{C}$  for further processing.

The industrial conjugation process was initially developed using tetanus toxoid by a team headed by J.B. Robbins at the National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Maryland.<sup>23</sup>

Conjugate preparation is a two-step process that involves: (a) activation of the Hib capsular polysaccharide and (b) conjugation of activated polysaccharide to tetanus protein through a spacer. Activation includes chemical fragmentation of the native polysaccharide to a specified molecular weight target and covalent linkage of adipic acid dihydrazide. The activated polysaccharide is then covalently linked to the purified tetanus protein by carbodiimide-mediated condensation using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. Purification of the conjugated material is performed to obtain high-molecular-weight conjugate molecules devoid of chemical residues and free protein and polysaccharide. Conjugate bulk is then diluted in an appropriate buffer, filled into unit-dose and/or multidose vials, and lyophilized.

## Live Attenuated Vaccine (Measles)

The measles virus, isolated in 1954, is part of the genus *Morbilivirus* in the family Paramyxoviridae. Current vaccines are derived from Edmonston, Moraten, or Schwarz strains. Such vaccines have been on the market since the 1960s and in combination (measles, mumps, rubella [MMR]) since the 1970s. The final vaccine is a live attenuated viral vaccine inducing immunity in more than 90% of recipients.

For one measles vaccine, the manufacture of the vaccine starts with specific pathogen-free embryonated chicken eggs that are incubated several days. The embryos are collected and treated with trypsin to prepare the chick embryo fibroblasts for cell culture. All of the operations are done under strict aseptic conditions, performed by well-trained operators.

Cell culture are grown in roller bottles using fetal calf sera and M199 Hanks media for optimal cell growth. Chick embryo fibroblast cells are further infected by the viral working seed and incubated several days for viral culture. At the end of the viral culture, the cells are disrupted by mechanical lysis to release the virus. The virus is purified by centrifugation and filtration and stored frozen. After release of all QC tests, the vaccine is formulated alone or with mumps and rubella vaccines and lyophilized to obtain the stable product. The vaccine is reconstituted just before use.

Other manufacturers use different cell substrates; for example, the Serum Institute of India uses human diploid cells to manufacture their measles vaccine (see [http://www.seruminstitute.com/content/products/product\\_mvac.htm](http://www.seruminstitute.com/content/products/product_mvac.htm)).

## Virus-Like Particle-Based Vaccines

Traditional viral vaccines rely on attenuated virus strains or inactivation of infectious virus. Subunit vaccines based on

viral proteins expressed in heterologous systems have been effective for some pathogens, but have often had poor immunogenicity because of incorrect folding or modification.<sup>24</sup> Virus-like particles (VLPs) are designed to mimic the overall structure of virus particles and, thus, preserve the native antigenic conformation of the immunogenic proteins. VLPs have been produced for a wide range of taxonomically and structurally distinct viruses and have unique *potential* advantages in terms of safety and immunogenicity over previous approaches.<sup>1</sup> Attenuation or inactivation of the VLP is not required; this is particularly important as epitopes are commonly modified by inactivation treatments.<sup>25</sup> However, if a viral vector (e.g., baculovirus) is used as the expression system, inactivation may be required if the purification process cannot eliminate residual viral activity.

For a VLP to be a realistic vaccine candidate, it needs to be produced in a safe expression system that is easy to scale up to large-scale production<sup>1</sup> and by an accompanying purification and inactivation process that will maintain native structure and immunogenicity and will meet the requirements of today's global regulatory authorities. A number of expression systems manufacture multimeric VLPs, including the baculovirus expression system (BVES) in Sf9 and High Five cells, *Escherichia coli*, *Aspergillus niger*, Chinese hamster ovary cells, human function liver cells, baby hamster kidney cells, transgenic plants (potato, tobacco, soybean), *S. cerevisiae*, *Pichia pastoris*, human embryonic kidney 293 (HEK293) cells, and lupin callus (a plant-cell production system) with yields ranging from 0.3 to 10  $\mu\text{g}/\text{mL}$  or as high as 300 to 500  $\mu\text{g}/\text{mL}$  with *E. coli* and HEK293 (purified).<sup>2</sup>

The BVES has proven quite versatile, demonstrating the capability of preparing vaccine candidates for papillomavirus, feline calicivirus, hepatitis E virus, porcine parvovirus, chicken anemia virus, porcine circovirus, SV40 (simian virus 40), poliovirus, bluetongue virus, rotavirus, hepatitis C virus, HIV, simian immunodeficiency virus, feline immunodeficiency virus, Newcastle disease virus, severe acute respiratory syndrome (SARS) coronavirus, Hantaan virus, influenza A virus, and infectious bursal disease virus.<sup>1</sup>

Many pathogenic viruses, such as influenza, HIV, and hepatitis C, are surrounded by an envelope, a membrane that consists of a lipid bilayer derived from the host cell, inserted with virus glycoprotein spikes. These proteins are targets of neutralizing antibodies and are essential components of a vaccine. Owing to inherent properties of the lipid envelope, assembly of VLPs in insect cells for these viral vaccines is a different type of technical challenge to those produced viruses with multiple capsids.<sup>1</sup> For these targets, production of VLPs is a challenging task because the synthesis and assembly of one or more recombinant proteins may be required. This is the case for VLPs of rotavirus, which is an RNA virus with capsids formed by 1860 monomers of four different proteins. In addition, the production of most VLPs requires the simultaneous expression and assembly of several recombinant proteins, which, in the case of RLP, needs to occur in a single host cell.<sup>26</sup> Purification of VLPs also constitutes a particularly challenging task. VLPs are structures of several nanometers in diameter and of molecular weights in the range of  $10^6$  Da. Also, for guaranteeing the quality of the product, it is not sufficient to demonstrate the absence of contaminant proteins; it is also necessary to show that proteins are correctly assembled into VLPs.

Production of HPV VLPs represents another challenge. The HPV type 16 major 55-kDa capsids protein, L1, when produced in certain recombinant expression systems such as *S. cerevisiae*, can form irregularly shaped VLPs with a broad size distribution. These HPV VLPs are inherently unstable and tend to aggregate in solution. The primary challenge of HPV vaccine

formulation development was the preparation of aqueous HPV VLP solutions that are stable under a variety of purification, processing, and storage conditions. By treating the HPV VLPs through a process of disassembly and reassembly, the stability and *in vitro* potency of the vaccine are enhanced significantly. In addition, the *in vivo* immunogenicity of the vaccine was also improved by as much as approximately 10-fold, as shown in mouse potency studies.<sup>27</sup> The disassembly and reassembly of particles may also be important to remove residual proteins from the expression system or host cells used in the production and is a serious processing challenge, particularly for enveloped VLPs.

## PRODUCT DEVELOPMENT

Vaccine development involves the process of taking a new antigen or immunogen identified in the research process and developing this substance into a final vaccine that can be evaluated through preclinical and clinical studies to determine the safety and efficacy of the resultant vaccine. During this process, the product's components, in-process materials, final product specifications, and manufacturing process are defined. The manufacturing scale used during development is usually significantly smaller than that used in the final manufacturing process. Phase I and, sometimes, Phase II clinical trial vaccines are typically produced in product development, but it is usually anticipated that at least one of the three or more consistency lots used for Phase III clinical trials will be manufactured at full-scale production volume. The product manufactured during the development phase is manufactured according to current GMP.<sup>28</sup>

## INDUSTRY'S RESPONSE TO NEW CHALLENGES

### Manufacturing Flexibility in Scale-Up: New Trends in Single Use

Early vaccines were produced *in vivo* (e.g., infecting calves with cowpox or rabbits with rabies virus; indeed, most influenza virus vaccines are still made in chicken embryos) and used neat or purified with largely glass lab equipment. Later, the animal cells used for viral vaccines were grown *in vitro* (e.g., roller bottles) and then infected to amplify virus for viral vaccines; later these cells were grown on microcarriers in deep culture to enable high manufacturing volumes and efficiencies. Microbial vaccines have been produced in large (500 to 5000 L) bioreactors at larger scale. These products were processed in stainless steel equipment for purification. Large-scale production was used to increase capacity to meet the increased demand for these lifesaving products. The cost of these facilities was increasingly expensive through the past few decades, and systems were increasingly complex to automate, clean, sterilize, and validate the facilities for manufacturing. Large central manufacturing facilities enabled lower cost of manufacturing; higher volumes were needed to lower the high fixed cost of construction, validation, and operation. The large central facilities were increasingly efficient, but also limited the manufacturing to a limited number of sites in developed countries.

Higher production yields and new manufacturing techniques, and an increasing focus on personalized medicine and niche products (e.g., low-volume products for rare diseases),<sup>29</sup> has allowed a new paradigm of smaller manufacturing facilities and bioreactors. The advent of disposable single-use equipment has eliminated the high cost of cleaning and sterilizing manufacturing equipment, reducing the complexity and cost of manufacturing facilities, and allowing economical

manufacturing at smaller scale. Manufacturing systems that were enabled by 100% single-use technology enabled competitive pricing and a significant time advantage in smaller facilities.<sup>30</sup> The promise of the technology is clear and has triggered a significant investment by equipment developers into the single-use space.

The single-use promise is significant; however, there are a number of hurdles as in any new technology format. Glass and stainless steel equipment of historical manufacturing processes is generally inert and do not contribute impurities to the manufacturing process. The polymers used in single-use equipment may have different reactivity with the product (extractables and leachables), have been shown to shed particulate, and have shown a tendency to leak. Many of these concerns are being overcome in time through polymer engineering, and altering production techniques. Next, the ability to eliminate the high cost of cleaning and sterilizing equipment requires that the entire system be single-use. Many processes have not been able to make this conversion because of yield (low yield means high volume; volumes >2000 L are challenging because of weight and hydrostatic pressure), pressure (high O<sub>2</sub> demand, aeration volume, or flow rate), solvent use, and temperature control (heat transfer limitations). Chromatography also has been a challenging process in single-use systems, although there are promising advances and increasing options for prepacked columns.<sup>29</sup>

With the promise and dynamic pace of advancement in single-use systems, manufacturers are faced with a plethora of options and a jigsaw puzzle of parts to connect effectively. As the industry is rather new, the standards necessary to allow interconnectivity of parts are still marginal but gaining attention.<sup>31</sup> Standards are in development for extractables and leachables, particulate classification and management, integrity, supplier evaluation, and interchangeability. Governing and sponsoring agencies include Parental Drug Associate, Bio-Process Systems Alliance, Extractables and Leachables Safety Information Exchange, Product Quality Research Institute, International Society of Pharmaceutical Engineers, American Society for Testing Materials, and United States Pharmacopeia, to name a few. Managing standards will be key to an effective use and integration of technologies supporting the vision of 100% single-use, reliable contingency supplies, robust supply chains, and limited surprises in change management.

### Promise and Challenges of Distributed Manufacturing

One major advantage of the right-sized, single-use process is the ability to support distributed manufacturing. Today, a vast majority of supply of vaccines comes from a handful of developed countries because of the cost and complexity of the large-scale facilities and the profitability of those markets that support the significant manufacturing and clinical investment of public companies necessary to license and manufacture a vaccine. Once a large facility is built, duplicating it is a significant challenge as the complexity of the support systems essentially makes every facility unique. The advent of low-capital, single-use manufacturing platforms supports "scale-out" of processes built with "off-the-shelf" single-use systems, truly duplicating the original licensed process and minimizing the challenge of providing equivalence of the product from new facilities to the original facility. One can also imagine making clinical materials at modest scale and avoiding the challenge of scale-up for all markets, which could leverage addition of multiple single-use bioreactors instead of a large stainless system as demand grows. The approach supports a "pay-as-you-go" capital approach, avoiding large investments prior to demonstrating proof of concept and has the promise to make

product available more broadly and more quickly after license through global partnerships and distribution of facilities nearer to the market they will serve. It is well aligned with the current developing policies of multiple emerging economies to be self-reliant for essential services like vaccine supply. For example, a 2009 agreement between GlaxoSmithKline and Brazil describes the sale of GlaxoSmithKline pneumococcal conjugate vaccine to Brazil for 8 years, with an agreement to transfer the manufacturing process to Brazil for potential use in future years.<sup>32</sup>

The distributed manufacturing approach has many benefits including limiting global supply shortages from a catastrophic event at a single plant, the ability to customize a product for a region (e.g., strain of organism changing by region), and offsetting some of the cost of manufacturing (potentially paid by government) with tax-generating local jobs. There are some challenges to be considered. Maintaining alignment of the process among multiple facilities can be challenging in biological manufacturing processes. A process can “drift” in any single plant; having multiple manufacturing plants increases the chances that at least one plant will drift from the licensed, proven process. Likewise, control of raw material quality and source, single-use component supply, changes in supplier’s materials in components, and similar unintended, unexpected consequences can leave a region without supply until the issue is identified and resolved. Single-use systems are ever improving; keeping the very specific needs of a process may be challenging with multiple small purchasers of the equipment versus a large central organization. Managing the regulatory files of a diverse production approach within a single company or across companies for a single product may also be difficult for regulators.

In spite of the challenges noted, the benefits of the distributed model, the advancement of standards within single-use manufacturers and the solutions that come with the experience of expanded use are sure to make this a reality in time.

## Future Challenges in Polio Vaccine Manufacturing

The manufacture of polio vaccines began in 1955, and ultimately resulted in two products that have been widely distributed: the Salk inactivated polio vaccine (IPV) and the Sabin live attenuated polio vaccine (OPV). The OPV is manufactured typically on a cell line with minimal purification postharvest. This combined with the relatively high productivity of doses/liter of capacity results in a lower cost of manufacture. Because the risk of vaccine-associated paralytic poliomyelitis (VAPP) is estimated at 0.42 per million<sup>33</sup> over a three-dose schedule, there is a planned transition to the Salk IPV as the polio comes closer to eradication. To ultimately make this switch, however, will require substantial increases in the capacity to manufacture IPV, and without productivity improvement, it would require substantial increases in the cost of delivering polio vaccines to the target populations.

The production of IPV as described in Table 5.1 shows that the virus must be grown in cell culture, and then purified using three chromatography steps, followed by inactivation. As with any bioprocess, each step results in loss of material that reduces the overall productivity. Alternatively, OPV is manufactured on cell lines with minimal purification. In addition, the dose of a live viral vaccine is typically much lower than an inactivated one, also helping the productivity of the process. This is reflected in the pricing of these vaccines. UNICEF publishes the prices of vaccine procured, and in 2014 OPV in a 20-dose vial from the Serum Institute of India was \$0.14/dose, whereas IPV from Bilthoven Biologicals/Serum Institute of India in a five-dose vial was \$1.90/dose.<sup>34</sup>

An additional challenge is the World Health Organization (WHO) guidelines on the use of wild-type polio virus necessary for the manufacture of IPV. Given the potential for an accident in manufacturing that could release wild-type poliovirus into the environment, the WHO has recommended limiting wild-type manufacturing of IPV to geographies with high IPV coverage in the surrounding community (>90% and at least three doses), and in environments with low transmission potential.<sup>35</sup> Currently, most of the vaccines for global vaccination come from India and China, where the suppliers of vaccines have focused on low-cost production and delivery of vaccines, and these geographies are currently not an option for IPV manufacture based on the WHO GAPIII recommendations.

A variety of strategies are currently being developed in the field to address these challenges, including a focus on the manufacturing technologies utilized to manufacture IPV, and opportunities to increase productivity and reduce unit costs. Existing polio vaccine manufacturing processes were developed years ago and use stainless steel fixed infrastructure manufacturing processes. Newer single-use technologies allow lower costs for initial facility design and could be used for polio vaccine manufacturing. An analysis by Lopes and colleagues shows that using newer facility design approaches that include single-use technologies can reduce bulk IPV costs by 40%, predominantly by reducing the capital costs of the facility.<sup>36</sup> Another approach to decreasing the cost is to increase the productivity of the manufacturing process.

Optimization of cell culture conditions and use of alternative cell lines are being evaluated, which suggest much higher titers of wild-type virus that could drive down the costs of the vaccine. Thomassen and colleagues showed that Vero cell culture can be further optimized to increase the production of D-antigen threefold.<sup>37</sup> Crucell has presented data that show its PER.C6 human-derived cell line has productivity 30 times higher than the Vero cells that are currently used to manufacture IPV.<sup>38</sup>

To allow broader manufacture of polio vaccines, an alternative vaccine using the Sabin attenuated strains but then inactivating them is also being pursued (Sabin-IPV [sIPV]). These viruses have shown lower yields in cell culture, but further work is being done to optimize their expression and purification yields.<sup>39,40</sup> This work again is focusing on cell-line selection, optimization, and facility design. Most importantly, the use of sIPV will allow a broader geographic set of potential locations for manufacturing, including India and China where there is substantial manufacturing infrastructure for vaccines focused on low-cost manufacturing.

Just as the initial discovery of polio vaccines required innovation in manufacturing methods to enable their launch, the ultimate eradication of polio will require innovation in manufacturing to enable the full public health impact of the vaccines.

## Prime-Boost Vaccines

Given the complexities of developing vaccines for difficult targets like HIV, tuberculosis (TB), and malaria, a new tool has emerged that uses a “prime-boost” strategy for vaccination. The concept uses one vaccine as a prime, followed by the use of an entirely different vaccine for the boost. Most notably, an HIV vaccine efficacy trial in Thailand had a positive result using this approach. The Thai trial primed with a recombinant canarypox vector and followed with a recombinant gp120 protein boost.<sup>41</sup> Neither the canarypox nor the recombinant protein had shown efficacy on its own, but when combined gave a positive result in the trial. This approach has been used

in additional HIV vaccine efficacy testing,<sup>42</sup> TB vaccines,<sup>43</sup> and malaria,<sup>44</sup> among others.

Several challenges arise in the use of prime-boost vaccines that will need to be overcome if they are to be used in future licensed vaccines for distribution. The first is the complexity of multivalent vaccines. To have a successful batch release, prime-boost vaccines, like multivalent vaccines require successful manufacture and release of multiple components. For example, the HIV vaccines developed by the Vaccine Research Center at the National Institutes of Health (NIH) for HIV and tested for efficacy contained six plasmid DNA components in the prime, and five recombinant adenovirus vectors in the boost.<sup>43</sup> This requires the production of 10 separate active pharmaceutical ingredients, which must then be combined into two separate formulations and tested. Failure of any one component will delay both the ability to combine materials and the manufacturing of the final products. Yields of individual components and consequent costs can vary widely, requiring unique manufacturing supply chain designs that provide consistent supply of the projected demands.

A critical component of assay development for any vaccine is the development of a potency assay for release. With two vaccines required for efficacy testing, defining exactly what the potency requirements are from each component will be important during clinical studies to ensure that future batches for distribution meet these criteria. It may require a prime-boost immunogenicity evaluation in animals for batch release, which has decreased with improved *in vitro* methods for potency evaluation of vaccines.

Finally, most of the prime-boost approaches often involve multiple organizations or companies. Most vaccine companies focus on a core set of vaccine platform technologies, which allow them to build expertise in process development, assay development, and manufacturing facilities. Nearly all of

the vaccines cited for HIV, TB, and malaria use products from separate organizations. Ultimately, if the vaccines prove successful, there needs to be a single regulatory license holder responsible for the distribution of the vaccine, which requires careful collaboration between the different organizations for licensure and life-cycle management of the vaccines.

Most of the prime-boost vaccines being tested are for early clinical trials that are designed to see an early efficacy signal from the vaccine. Given this, there is limited focus on the future manufacturing supply chain and production costs. Even at these early stages of clinical studies, however, it is important to ensure partnerships are clearly defined among vaccine developers, and the clinical studies are designed to demonstrate the true requirement for the prime-boost regime and the characteristics that each component must elicit after immunization. Early cost modeling can help target investments in process development to ensure that a successful signal in efficacy testing can ultimately result in a vaccine that can be successfully manufactured and distributed to the target populations.

A final challenge will be the coordination of the supply chains. As two separate vaccines are required for complete immunizations and potentially different immunization times, deployment and supply of the vaccines have to be synchronized to demand. For routine immunizations this is relatively straightforward; but for any catch-up or mass vaccination campaigns, it requires care management of expiration dates and planning. Vaccine supply shortages are unfortunately not uncommon, and a requirement for multiple vaccines increases the possibility that one might not be continuously available, creating partially immunized and potentially off-schedule immunized individuals. The implications of these scenarios may have to be explored during Phase II dosing studies or postmarketing surveillance.

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